Appendix I: Alignment of SEQ ID NO: 7 with the chloramphenicol resistance gene of Borges et al.

<!--StartFragment-->RESULT 3
AAS00462
ID AAS00462 standard; DNA; 2003 BP.
XX
AC AAS00462;
XX
DT 11-JUN-2007 (revised)
DT 16-MAY-2001 (first entry)
XX
DE Plasmid pLOI2225 useful for chromosomal integration of heterologous DNA.
XX
KW Plasmid; vector; antibiotic resistance; ethanol; alcohol dehydrogenase;
KW adhB; pyruvate decarboxylase; pdc; chloramphenicol acetyl transferase;
KW cat; regulatory element; adhE; chromosomal integration; circular; cyclic;
KW pLOI2225; pLOI2222; pSG76-C; chloramphenicol resistance;
KW FRT recombining site; ds.
XX
OS Synthetic.
XX
FH Key Location/Qualifiers
FT CDS complement(1045. .1704)
FT /*tag= a
FT /note= "Chloramphenicol-resistance gene"
XX
PN WO200118222-A1.
XX
PD 15-MAR-2001.
XX
PF 18-AUG-2000; 2000WO-US022700.
XX
PR 07-SEP-1999; 99US-00390479.
XX
PA (UYFL) UNIV FLORIDA.
XX
PI Borges AC, Zaldivar J, Morales FM, Jimenez AM, Ingram LO;
XX
DR WPI; 2001-235205/24.
DR PC:NCBI; gi6467484.
XX
PT Novel nucleic acid construct for integrating heterologous nucleic acid
PT sequences into genome or chromosome of host cells, has passenger and
PT marker sequences, in which marker sequence is flanked by recombining
PT sites.
XX
PS Claim 28; Page 59-60; 85pp; English.
XX
CC The present sequence for plasmid pLOI2225 which is constructed from the
CC plasmids pLOI2222 and pSG76-C is 1 of 7 novel plasmid constructs
CC (AAS00460-AAS00466) comprising a marker sequence such as an antibiotic
CC resistance gene, in which the marker sequence is flanked by two FRT
CC recombining sites. One of these plasmids (pLOI02231) also comprises a
CC passenger sequence. The passenger sequence can include an ethanologenic
CC gene such as alcohol dehydrogenase (preferably adhB) or pyruvate
CC decarboxylase (pdc), another gene such as chloramphenicol acetyl
CC transferase (cat), a regulatory element such as a promoter or IRES
CC (internal ribosomal entry site) or a guide sequence such as adhE. All the
CC plasmids are useful for integrating a nucleic acid construct into the
CC genome of a cell. Plasmid pLOI02231 is useful for producing ethanol by,
CC transforming an ethanologenic cell with the plasmid and contacting the
CC cell with a substrate which can be fermented into ethanol, where
CC expression of the passenger sequence results in the production of

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CC   ethanol. The recombinant ethanologenic host transformed with the plasmid
CC   has improved properties including increased ability to produce ethanol,
CC   depolymerisation for a particular substrate and increased tolerance to a
CC   higher level of ethanol
CC
CC   Revised record issued on 11-JUN-2007 : Enhanced with precomputed
CC   information from BOND.
XX
SQ   Sequence 2003 BP; 558 A; 425 C; 436 G; 584 T; 0 U; 0 Other;

  Query Match             100.0%;  Score 1069;  DB 1;  Length 2003;
  Best Local Similarity   100.0%;  Pred. No. 0;
  Matches 1069;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 GCAAAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTG 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        935 GCAAAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTG 994

Qy         61 ACAGTTACCAATGCTTAATCAGTGAGGCACCAATAACTGCCTTAAAAAAATTACGCCCCG 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        995 ACAGTTACCAATGCTTAATCAGTGAGGCACCAATAACTGCCTTAAAAAAATTACGCCCCG 1054

Qy        121 CCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCA 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1055 CCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCA 1114

Qy        181 TCACAGACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTA 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1115 TCACAGACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTA 1174

Qy        241 TAATATTTGCCCATGGTGAAAACGGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAA 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1175 TAATATTTGCCCATGGTGAAAACGGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAA 1234

Qy        301 TCAAAACTGGTGAAACTCACCCAGGGATTGGCTGAGACGAAAAACATATTCTCAATAAAC 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1235 TCAAAACTGGTGAAACTCACCCAGGGATTGGCTGAGACGAAAAACATATTCTCAATAAAC 1294

Qy        361 CCTTTAGGGAAATAGGCCAGGTTTTCACCGTAACACGCCACATCTTGCGAATATATGTGT 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1295 CCTTTAGGGAAATAGGCCAGGTTTTCACCGTAACACGCCACATCTTGCGAATATATGTGT 1354

Qy        421 AGAAACTGCCGGAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGC 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1355 AGAAACTGCCGGAAATCGTCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGC 1414

Qy        481 TCATGGAAAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTC 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1415 TCATGGAAAACGGTGTAACAAGGGTGAACACTATCCCATATCACCAGCTCACCGTCTTTC 1474

Qy        541 ATTGCCATACGGAATTTCGGATGAGCATTCATCAGGCGGGCAAGAATGTGAATAAAGGCC 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1475 ATTGCCATACGGAATTTCGGATGAGCATTCATCAGGCGGGCAAGAATGTGAATAAAGGCC 1534

Qy        601 GGATAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGA 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1535 GGATAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGA 1594

Qy        661 ACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGA 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1595 ACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGA 1654
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Qy        721 TGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTTAGCTTCC 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1655 TGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCCATTTTAGCTTCC 1714

Qy        781 TTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCATTA 840
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1715 TTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCATTA 1774

Qy        841 TGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTTGGCC 900
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1775 TGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTTGGCC 1834

Qy        901 CAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTCTGCGAAGTGATCTTCC 960
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1835 CAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTCTGCGAAGTGATCTTCC 1894

Qy        961 GTCACAGGTATTTATTCGGCGCAAAGTGCGTCGGGTGATGCTGCCAACTTACTGATTTAG 1020
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1895 GTCACAGGTATTTATTCGGCGCAAAGTGCGTCGGGTGATGCTGCCAACTTACTGATTTAG 1954

Qy       1021 TGTATGATGGTGTTTTTGAGGTGCTCCAGTGGCTTCTGTTTCTATCAGC 1069
              |||||||||||||||||||||||||||||||||||||||||||||||||
Db       1955 TGTATGATGGTGTTTTTGAGGTGCTCCAGTGGCTTCTGTTTCTATCAGC 2003

<!--EndFragment-->
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